BREEDING SYSTEMS, POPULATION STRUCTURE, AND EVOLUTION IN HYDROPHILOUS ANGIOSPERMS[1]

Donald H. Les[2]

ABSTRACT

True hydrophily occurs in 18 submersed angiosperm genera. Of these, 17 are monocots, 12 are marine, and 5 contain annuals. Three genera include hermaphroditic species, 8 have monoecious species, and 13 have dioecious species. The prevalence of dicliny in hydrophiles has led to assumptions of outcrossing and high levels of genetic variability in these plants. Many water-pollinated species, however, may often fail to meet all conditions necessary for outcrossing. In hydrophiles, decreased seed output associated with dicliny increases the probability of dispersal by vegetative propagules. The predominant role of asexual reproduction and clonal growth in many hydrophile populations may restrict outcrossing despite the high potential for xenogamy in some species. This inference is substantiated by preliminary genetic analyses which indicate very low levels of variability and high homozygosity in hydrophile populations. A shift to asexual reproduction is offered as one explanation for the slow evolutionary rates associated with this biological group of species. Exceptions to these conclusions are noted in annual species, which must reproduce sexually to survive. Annual hydrophile genera have higher species diversity and possibly greater genetic variability within and between populations.

Aquatic plants are notorious for their anatomical, morphological, and physiological peculiarities. A question relating to their unusual biological features is whether the course of evolution in aquatic plants differs fundamentally from that of terrestrial plants. This question is difficult to address because water plants are not a monophyletic group; therefore, the assessment of "peculiarities" would necessarily involve interpreting patterns of convergent evolution. Certainly, there is no reason to suspect that hydrophytes do not follow evolutionary paths dictated by the same basic factors that have influenced terrestrial plant evolution. On the other hand, there are evolutionary patterns associated with the "biological group" of submersed hydrophilous angiosperms that warrant consideration. A particularly striking feature of these species is their slow rate of evolutionary diversification, a conclusion revealed by several lines of evidence.

Data tabulated for 31 principally aquatic families (from Cook et al., 1974) furnish an average of about eight species per genus. The number of extant species in most hydrophilous angiosperm genera, however, is fewer than eight (Table 1). In comparison, some genera of nonhydrophilous submersed aquatic plants (e.g., *Potamogeton, Myriophyllum, Ranunculus*) may contain 35–100 or more species. Not all hydrophilous genera, however, have few species; *Najas* contains 35–50 species, the largest number among hydrophilous genera (Table 1). Furthermore, the fossil record indicates that at least several hydrophilous angiosperms have undergone a prolonged period of morphological "stasis." In an evolutionary study of the genus *Ceratophyllum*, Les (1986a: 32) observed: "One interesting aspect of the fossil record is that most of the extinct taxa can be associated with the extant genus *Ceratophyllum*, and often with extant species . . . ." Similar statements were made by Hartog (1970) with respect to the hydrophilous seagrasses, e.g. (p. 15): "It is noteworthy that these Tertiary [seagrass] fossils all belong to still existing genera and that at least two of them can be identified with still existing species." And (p. 30): ". . . evidence for the great age of recent species . . . is supported by the fact that fossil remains of *Cymodocea* from the European Eocene can be identified with still existing species." Hartog (1970)

TABLE 1.   *Synopsis of angiosperm genera with submersed hydrophilous species. H = hyphydrophilous; E = ephydrophilous; Mo = monoecious; D = dioecious; Hm = hermaphroditic; P = perennial; A = annual; M = marine; B = brackish; F = fresh (compiled from Aston, 1973; Cook, 1982; Cook et al., 1974; Cox, 1983; Edwards, 1976; Hartog, 1970; Haynes, 1977; Haynes & Holm-Nielsen, 1987; Hutchinson, 1975; Les, 1986a; Sculthorpe, 1967).*

	Number of Species	Sexual Reproduction	Sexual Condition	Life Form	Habitat	Pollen
DICOTYLEDONS						
Ceratophyllaceae						
Ceratophyllum (H)	6	rare to common	Mo	P	B, F	precocious
MONOCOTYLEDONS						
Hydrocharitaceae						
Elodea (E)	5	rare	D, Mo	P	F	globose
Halophila (H)	8	rare to common	D, Mo	P	M	chains & precocious
Thalassia (H)	2	rare to common	D	P	M	chains & precocious
Najadaceae						
Najas (H)	35–50	prolific	Mo, D	A, P	B, F	precocious
Posidoniaceae						
Posidonia (H)	3	rare to common	Hm	P	M	filiform
Ruppiaceae						
Ruppia (E)	1–7	common	Hm	A, P	M, B, F	chains
Zosteraceae						
Heterozostera (H)	1	common	Mo	P	M	filiform
Phyllospadix (H)	5	common	D	P	M	filiform
Zostera (H)	12	rare to common	Mo	A, P	M	filiform
Zannichelliaceae						
Althenia (H)	2	common	D	P	B, F	globose
Amphibolis (H)	2	common	D	P	M	filiform
Cymodocea (H)	4	rare	D	P	M	filiform
Halodule (H)	6	rare	D	P	M	filiform
Lepilaena (E, H)	4	common	Mo, D	A, P	B, F	globose
Syringodium (H)	2	common	D	P	M	filiform
Thalassodendron (H)	2	infrequent	D	P	M	filiform
Zannichellia (H)	1–5	common	Mo, D, Hm	A, P	B, F	globose & precocious

attributed the slow rate of evolution in seagrasses to the relative uniformity of the marine environment, and Les (1986a) related stasis in *Ceratophyllum* to interactions of hydrophily and aspects of the breeding system.

Are the slow evolutionary rates that apparently characterize various unrelated hydrophile species a consequence of their unique pollination system? This question has prompted the present study to review not only the pollination system but the overall reproductive biology of hydrophilous angiosperms. Specifically, the intent of this paper is to hypothesize possible evolutionary implications associated with peculiarities of hydrophile reproductive biology. Establishment of a theoretical basis

will provide a means for testing hypotheses empirically. Although this symposium focuses on freshwater angiosperms, consideration must also be given here to marine angiosperms, which dominate this biological group.

SALIENT FEATURES OF HYDROPHILY

True hydrophily includes hyphydrophily where pollen is transported exclusively under water, and ephydrophily where pollination occurs at the surface (Faegri & van der Pijl, 1979). Various mechanisms by which pollen is transported above the water surface (e.g., *Enhalus*, *Vallisneria*) mimic hydrophily but are not considered here.

Hydrophily is viewed as a derived condition in angiosperms and probably developed from both anemophily and entomophily (Faegri & van der Pijl, 1979); however, the immediate precursor to hydrophily in most instances appears to have been anemophily (Les, 1988b). Hydrophily is unique to submersed aquatic angiosperms and occurs only within 18 genera which represent seven families and which constitute a heterogeneous group phylogenetically. The taxonomic distribution and selected features of these genera are summarized in Table 1, from which several associations are apparent. Except for *Najas*, there are 1–12 species in hydrophilous genera. The frequency of sexual reproduction ranges from common to rare. Nearly all genera are principally perennial, and all (even annuals) possess mechanisms for vegetative reproduction. An overwhelming consistency is the diclinous sexual condition (monoecy or dioecy), with hermaphroditic flowers occurring in only three genera. Most hydrophilous genera are marine; seven genera occur in freshwater.

Like anemophily, hydrophily is an abiotic pollen-transfer mechanism and therefore inherently "wasteful" (i.e., much of the pollen produced does not contact a receptive stigma) because of nondirectionality (Faegri & van der Pijl, 1979; Cox, 1983). The three-dimensionality of hyphydrophily leads to high pollen wastage, although higher efficiency may be attained in shallow water where pollen loads may concentrate. Ephydrophily reduces pollen wastage by confining the dispersal of grains to the two-dimensional water surface (Faegri & van der Pijl, 1979). Because of the stochastic nature of hydrophily, the highest level of efficiency is probably achieved with autogamous pollinations, where the shortest transport distance is involved, and presumably decreases with the greater distances involved in geitonogamous (involving different flowers on one individual) or xenogamous (involving flowers on different individuals) pollinations. Underwater pollination in some species is enhanced by the reduction of water currents over plant beds (which assists pollen deposition) and the relatively large area of pollen influence around female flowers (Ackerman, 1983, 1986).

Hydrophile pollen exhibits structural modifications that apparently maximize transport efficiency. The surface area of most hydrophile pollen is increased variously (Table 1). In some species, globular pollen grains form filiform chains, or the grain itself is highly elongate (Pettitt & Jermy, 1975; Cox, 1983). In other species, precocious germination of the pollen tube (Fig. 1A) may increase surface area to maximize capture by the stigma (Sculthorpe, 1967; Faegri & van der Pijl, 1979; Cox, 1983; Les, 1986a). In *Ceratophyllum*, the surface area is increased further by the occasional branching of pollen tubes (Fig. 1B), and grains with precocious pollen tubes have been observed to mass together (Sehgal & Ram, 1970), thereby mimicking pollinia. Typically, the exine of hydrophilous pollen is highly reduced (Pettitt & Jermy, 1975).

Hydrophily and anemophily are the major abiotic pollination systems in plants. Understandably, the two systems share certain similarities in their floral syndromes, such as frequent dicliny, reduced perianths, high pollen/ovule ratios, reduced pollen exines, and enlarged receptive surfaces (Faegri & van der Pijl, 1979; Les, 1986a; Sculthorpe, 1967; Whitehead, 1969). Both systems lack the specificity and constancy associated with biotic pollination systems. There are, however, various differences between the two abiotic systems. Anemophily relies on the availability of wind, whereas availability of water is seldom a liability to submersed plants. Pollen dispersal distances of anemophiles are limited only by the longevity of grains and their ability to be carried aloft. Dispersal distances in hydrophiles are restricted entirely to the dimensions of the body of water they inhabit. For freshwater species, this distance may be quite small, e.g., a pond, pool, or small lake. A further consideration is that hydrophiles are generally confined to shallow depths, and pollen transported to deeper water has little chance of encountering plants. With respect to ephydrophily, Cook & Urmi-König (1985: 118) stated: "Pollen transfer on the surface of the water may well be efficient over distances best measured in centimeters, but for distances measured in meters or kilometers it will become inefficient or even ineffective."

Hydrophile pollen is not subject to desiccation, but studies are needed to determine the duration of its viability. In *Zostera*, pollen retains viability for over 48 hours (De Cock, 1980). Although pollen of hyphydrophiles frequently contains starch to promote sinking (Sculthorpe, 1967; Les, 1986a), it is conceivable that water currents may facilitate resuspension of grains (particularly in very shallow water), which may increase opportunities for contact with submerged receptive stigmas.

A HYPOTHESIS OF OUTCROSSING IN HYDROPHILES

Most angiosperms are hermaphroditic (Bawa & Beach, 1981), with dioecy (depending on the region) occurring in only 2–28% of species (Bawa, 1980) and monoecy equally rare (Grant, 1975).
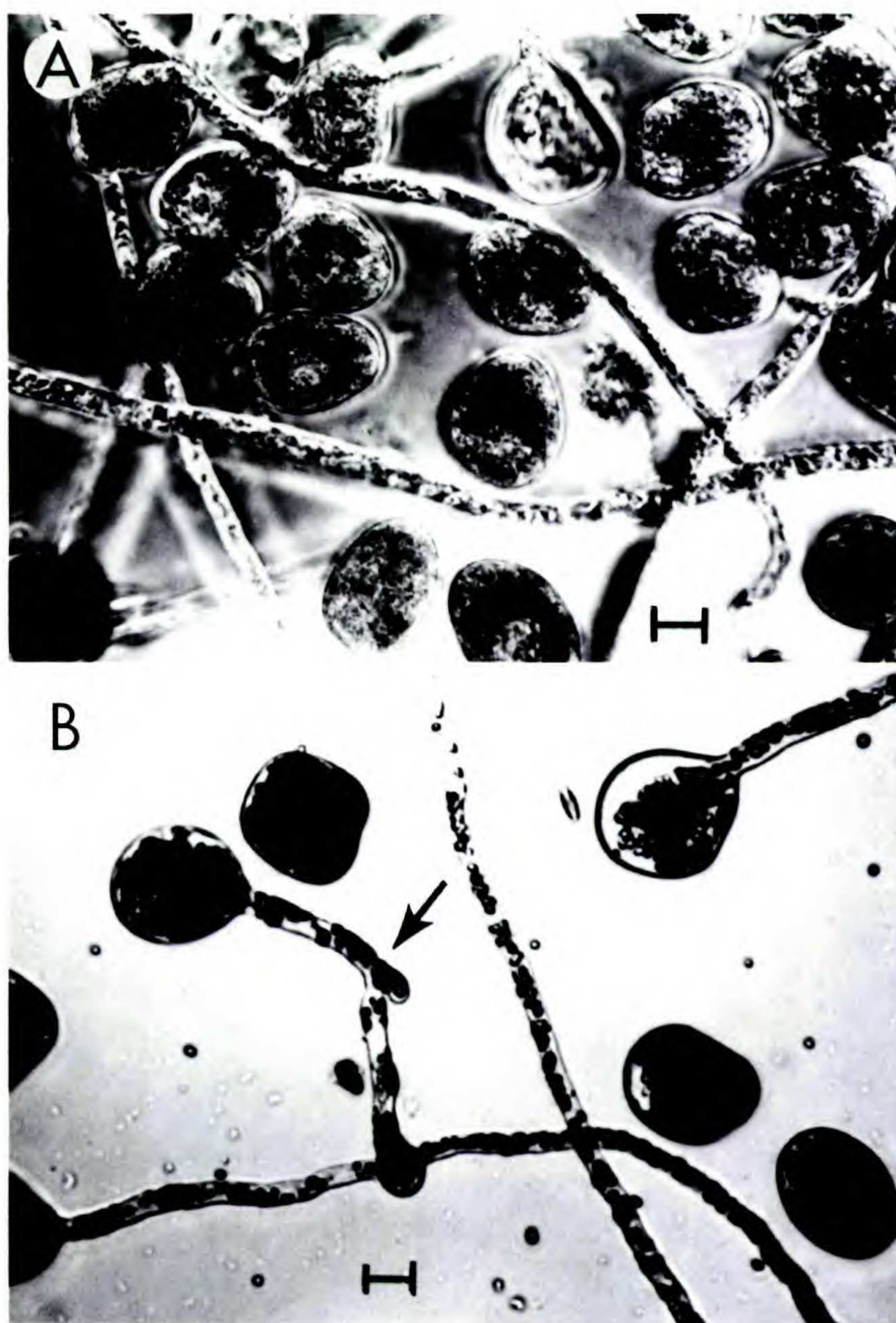
FIGURE 1. *Pollen tube modifications in* Ceratophyllum demersum. —*A. Precocious germination of grains showing elongated pollen tubes.—B. Branching of pollen tubes (at arrow). Scale bars = 5 μm.*

In contrast, 13 of the 18 genera (72%) of hydrophilous angiosperms have dioecious species; 8 genera (44%) have monoecious representatives; and only about 8.7% of hydrophile species are hermaphroditic (Table 1). The association between dioecy and hydrophily was noted by Sculthorpe (1967), who did not provide an evolutionary explanation for the co-occurrence of these traits.

Others, however, have viewed the predominance of dicliny in hydrophiles as evidence of an inevitable association with outcrossing and production of genetically variant progeny (Hartog, 1970; Pettitt et al., 1981). Although dioecy is associated with reduced prolificity because of the presence of males (Lloyd, 1980), it has been postulated (in seagrasses) that "... gains in survival attributable to continuous out-breeding outweigh the disadvantages of diminished seed production" (Pettitt et al., 1981: 137). These conclusions can be interpreted as a

hypothesis that embraces three components: 1) hydrophiles are characterized by outcrossing, which leads to the production of genetically variable offspring; 2) the selective advantage of outcrossing in hydrophiles offsets the evolutionary costs associated with transitions to dicliny; and 3) dicliny has evolved in hydrophiles as a mechanism for promoting outcrossing. The appealing rationality of such a hypothesis belies the fact that it rests entirely on circumstantial evidence associated with sexual conditions and is not substantiated by more empirical evidence. Furthermore, it is difficult to rationalize the slow evolutionary diversification of this group with a supposed history of outcrossing and prolific genetic variability. It is possible that few species have made the transition to hydrophily in recent time, and therefore the hydrophile genera have simply not had sufficient time to diversify. This interpretation, however, is incongruent with

fossil evidence that indicates a great age of many hydrophile genera. Obviously, it is necessary to consider other sources of data that may be more pertinent in assessing the extent of outcrossing in hydrophiles.

TESTING THE "OUTCROSSING HYPOTHESIS"

In hydrophiles, the possibility of outcrossing (natural crossing as defined by Grant, 1975) exists only when several conditions are satisfied. One requirement is for sexual reproduction. A second requirement is for xenogamy, which assures that sperm of one individual reach the eggs of different individuals. Thirdly, the parents contributing gametes must differ genetically and their offspring must survive. By evaluating the ability of hydrophiles to satisfy these conditions, it may be possible to assess better the role of outcrossing in these species. To interpret fully the significance of outcrossing in hydrophiles, it is also necessary to understand the relative level of inbreeding that may occur in outcrossing species.

THE EXTENT OF SEXUAL REPRODUCTION IN HYDROPHILES

There has been much discussion regarding the costs and benefits of sexuality in organisms. Using a group selection model, Lloyd (1980) argued that dioecious populations experience a cost of sex, whereas hermaphroditic populations do not. Furthermore, he pointed out that asexual reproduction results in greater prolificity than dioecy, raises the potential rate of increase, and may aid in the long-term persistence of asexual populations and species. He concluded, however, that asexuality does not appear to have been significant in group selection against dioecy because of the relatively lower evolutionary success of asexual lines. Nevertheless, hydrophyte reproduction occurs both sexually and asexually, most species have well-developed means of vegetative reproduction, and most reproduction in perennial hydrophytes is estimated to be asexual (Hutchinson, 1975).

Approximately half of hydrophile genera are characterized by rare sexual reproduction (Table 1). A quote from Hartog (1970: 34) illustrates this point: ". . . inflorescences, flowers, fruits and seeds . . . are not often found in most seagrasses, and in some species they are not known at all or only incompletely." The rarity of fruiting in the freshwater genera Elodea and Ceratophyllum is also widely recognized (Cook & Urmi-König, 1985; Les, 1986a).

One facet of sterility in dioecious hydrophiles is the low percentage of flowering in several species. The seagrasses Halophila stipulacea, Halodule beaudettei, H. bermudensis, H. ciliata, Cymodocea rotundata, C. serrulata, C. angustata, and some Posidonia species are rare-flowering according to Hartog (1970). McMillan (1976, 1979, 1980) noted that environmental conditions have wide effects on the reproductive biology of many seagrasses. Even when flowering, dioecious hydrophiles may exhibit another facet of sterility. In Thalassia testudinum, fewer than 1% of plants may flower simultaneously in "beds," and beds are often unisexual (Hartog, 1970; Durako & Moffler, 1987). Such conditions may result in frequent sterility of the species, such as that reported by Edwards (1976). Similarly, plants of Thalassodendron ciliatum and Elodea species typically exist in unisexual colonies with plants of both sexes rarely coexisting (Hartog, 1970; Cook & Urmi-König, 1985). Such circumstances surely contribute to low fruit production in these species. Cook & Urmi-König (1985) attributed the unisexuality of Elodea populations to differential competition for habitat by the sexes. Alternate explanations, however, include the possibility that unisexual populations are derived clonally, or that sex expression is affected by environmental rather than by strictly genetic factors.

A further restraint of sexuality is the rarity of seedling production in some plants with high seed output, e.g., Zostera noltii (Hartog, 1970).

Agamospermy is possibly mistaken at times for sexual reproduction in some hydrophiles. In Halophila stipulacea, cultured plants induced to flower produced no males, yet the females produced seed (McMillan, 1980), a good reason to suspect agamospermy. In H. hawaiiana, male and female plants are not known to coexist (Herbert, 1986). Although agamospermous reproduction is genetically equivalent to vegetative propagation, one obvious difference is production of fruits allowing for "normal" dispersal. It would be an important contribution to test experimentally for agamospermy among other hydrophilous species.

Although gene recombination via sexual reproduction is viewed as important for response to changing or heterogeneous environmental conditions, genetic uniformity enforced by vegetative reproduction may be more advantageous for a species already adapted to uniform habitat conditions (Grant, 1981; Lloyd, 1980). Therefore, the relative uniformity of freshwater and marine environments (Hartog, 1970; Tiffney, 1981) provides one explanation for the ubiquity of efficient vegetative reproduction in hydrophytes.

Demands on parental energy for reproductive effort have been implicated in compromises between sexual and asexual avenues in some plants. In many instances, the relationship is inverse, i.e., high fruit production with low vegetative propagation and vice versa (Salisbury, 1942; Harper, 1977). In hydrophiles, free-fruiting species often exhibit less vegetative development than rare-fruiting species. Of the freshwater hydrophiles, poor vegetative development occurs in the genera *Althenia, Lepilaena, Ruppia, Najas,* and *Zannichellia,* which are all typically free-fruiting (Table 1). Of these genera, *Najas, Zannichellia,* and *Lepilaena* are mainly annual. In *Ruppia,* little biomass is allocated to reproductive structures in perennial species, whereas allocation to reproductive structures in annuals is always much higher (Brock, 1982). The freshwater genus *Elodea* has coarse vegetative growth and low seed output. All species of *Ceratophyllum* are perennial, yet much higher seed output occurs in species with fine foliage than in the coarse-leaved species *C. demersum* (Les, 1986a). Sometimes in *Zannichellia* relatively robust plants have been associated with lower flower and fruit production and behave like perennials, whereas slender plants have higher flower and fruit production (Uotila et al., 1983). Similar associations are not as evident in marine hydrophiles, possibly because a well-developed vegetative system for anchoring plants against forces of tidal currents and wave action is essential for marine existence (Hartog, 1970). In the marine genus *Zostera,* however, a relationship exists between fruiting and shoot development. Arber (1920: 127) observed: "In *Zostera marina* . . . the fertile and sterile plants are readily distinguishable from one another, since in the fertile plant the stem is slender, erect, and much branched, while that of the sterile individual is thick, creeping, more luxuriantly leafy, and anchored to the soil by adventitious roots . . . ." Furthermore, annual individuals of *Zostera marina* can be distinguished readily from perennial individuals by the former's lack of vegetative shoots and rhizomes (Keddy & Patriquin, 1978). Although most species within the principally annual genus *Najas* have relatively fine foliage, the perennial species *N. marina* is characterized by very coarse leaves and has the ability to form vegetative turions (Agami et al., 1986).

Differential resource allocation to reproduction has been studied in terrestrial plants in some detail (Silvertown, 1982) and provides a convenient explanation for the association of high vegetative development with low sexual reproduction in some hydrophytes. It is possible that the development of effective perennating mechanisms in hydrophytes was accompanied by a sacrifice of sexual reproduction. It could be argued that vegetative growth and reduced reproductive effort in perennials vs. high sexuality and reproductive effort in annuals are merely adaptive life history traits. On the other hand, the shoot dimorphism described above between both fertile/sterile and annual/perennial individuals of *Zostera marina* indicates that tradeoffs in reproductive allocation do occur. In *Zostera marina,* the different reproductive strategies (annual, perennial, sexual, asexual) are employed depending upon environmental circumstances (Phillips et al., 1983). Although other factors may be involved, it is possible that the relationship between low sexual reproduction and high vegetative development noted in hydrophiles and other hydrophytes is due in part to constraints related to resource allocation.

From the above discussion, it is evident that sexual reproduction in water-pollinated plants is not commonplace. This conclusion is important because, despite any other factor, outcrossing cannot occur in hydrophile populations that reproduce only asexually.

THE EXTENT OF XENOGAMY IN HYDROPHILES

Hermaphroditic, monoecious, and dioecious sexual conditions in hydrophiles allow for three possible means of gametic exchange: autogamy, geitonogamy, and xenogamy. Although outcrossing can occur only when gametic exchange is xenogamous, xenogamy is possible with all three sexual conditions.

Autogamy in many hermaphroditic terrestrial plants is prevented or restricted by mechanisms such as dichogamy, herkogamy, heteromorphy, and self-incompatibility (Faegri & van der Pijl, 1979; Lewis, 1979). Heteromorphy is associated with biotic pollinators (Lewis, 1979) and is unknown in hydrophiles. Incompatibility mechanisms have not been reported in hydrophiles, presumably because of constraints imposed by the water-liability of recognition substances and by reduction of the exine in hydrophilous pollen (Pettitt & Jermy, 1975). Herkogamy, the spatial separation of sexes, is not characteristic of hermaphroditic hydrophiles. This leaves only the possibility of dichogamy, the temporal separation of sexes, as a means of preventing autogamy in hermaphroditic hydrophiles.

Autogamy is possible only in three genera (*Posidonia, Ruppia, Zannichellia*) which have members with hermaphroditic flowers. Sexual reproduction in these genera is common (Table 1). Aston

(1973) described fruit production as "prolific" for *Posidonia*, and the numerous fruits of *Ruppia* and *Zannichellia* taken from waterfowl stomachs (up to 4,000 and 10,000 per stomach, respectively) indicate high fruit production in these genera (McAtee, 1939). A single plant of *Zannichellia* is capable of producing more than two million seeds in six months (Yeo, 1966). Discounting apomixis, the prolific fruit production in these genera reflects their successful adaptation to hydrophily. Much of this prolificity, however, probably results from autogamy.

In hermaphroditic *Zannichellia* (Aston, 1973), autogamy is possible due to the enclosure of stamen and carpels within the cuplike perianth, although the flowers may be dichogamous. Most species of *Zannichellia*, however, are monoecious and even some dioecy has been reported (Muhlberg, 1982). In monoecious *Zannichellia*, the arrangement of flowers results in functional bisexuality. Male and female flowers are typically adjacent and appear ". . . to rise as a group in a leaf axil" (Sculthorpe, 1967: 298). Although the anther of the male flower is raised above the carpels, pollen grains have a higher specific gravity than water and ". . . sink on to the peltate or tongue-shaped stigmas . . ." (Sculthorpe, 1967: 299). Arber (1920: 71) reported a similar scenario for *Zannichellia*. She noted that, "The anther dehisces and the pollen grains fall into the open mouths of the cornucopia-shaped stigma . . . ." These accounts suggest that dichogamy does not occur in *Zannichellia* and that selfing would be commonplace. Hutchinson (1975: 232), however, presumed that local turbulence would displace the descending pollen of *Zannichellia*, ". . . so that occasional cross-pollination can occur even when . . . the male and female flowers [are] very close together." The description of pollination in *Zannichellia* by Haynes & Holm-Nielsen (1987: 264) renders Hutchinson's presumption untenable: ". . . the anther of the staminate flower arches over the funnel-shaped stigmas of the carpellate flower. Pollen transfer is entirely underwater: it is released from the anther in a gelatinous mass and falls directly into the stigma." Although monoecious *Zannichellia* cannot be categorized as autogamous, these descriptions of pollination in the genus indicate predominant geitonogamy (virtually the genetic equivalent of autogamy). Even the effects of turbulence that Hutchinson proposed would probably result in geitonogamy rather than xenogamy, as the gelatinous pollen masses would be more likely to settle than to be transported laterally. Furthermore, the floral morphology of *Zannichellia* does not appear to be adapted for xenogamy. Pollen/ovule ratios are not known specifically in *Zannichellia*; however, they are probably relatively low, with only a single stamen for every cluster of four one-ovuled female flowers. The sculptured exine of *Zannichellia* pollen is atypical of hydrophilous angiosperms (Pettitt & Jermy, 1975). The pollen shape is globular and the grains do not form chains (Table 1), an indication that it is not as well-adapted for transport over distances as that of other hydrophiles. For *Zannichellia*, the high percentage of fruiting is likely a result of autogamy in hermaphroditic plants or of geitonogamy in monoecious plants, rather than of xenogamy.

Exceptions may occur with *Zannichellia contorta* and *Z. peltata*, in which the anther filaments are much longer than those of other species, and their pistillate and staminate flowers arise at different nodes (Talavera et al., 1986; Van Vierssen & Van Wijk, 1982). With the greater spatial separation of anthers and pistillate flowers in these species, the opportunity for xenogamy is enhanced. Haynes & Holm-Nielsen (1987) concluded that generally the pollination system in *Zannichellia* limits outcrossing but is valuable for the annual habit because pollination is essentially assured.

The pollination system of *Ruppia* has been described in some detail. In *Ruppia cirrhosa*, the hermaphroditic-flowered inflorescence reaches the surface of the water but remains submersed; the anthers dehisce, are carried to the surface by air bubbles, and release pollen explosively when contacting the atmosphere. Cohering pollen grains cover the surface in chainlike strings and eventually reach the carpels, which are raised to the surface by bending of the inflorescence. In *R. maritima*, the discoid, peltate stigmas form a canopy above the anthers and trap the ascending pollen chains; the grains drift around the stigma to its surface, where some adhere and germinate. (For details see Arber, 1920; Gamerro, 1968; McCann, 1945; Sculthorpe, 1967.)

In *Ruppia* it is difficult to ascertain the extent of autogamy. Frequent fruit production can be explained either by autogamy (such as described for *R. maritima* above) or by xenogamy. The observance of spreading surface pollen masses of *Ruppia* (Faegri & van der Pijl, 1979) demonstrates the potential for xenogamy. The two-dimensional nature of the ephydrophilous mechanism "concentrates" the pollen, thereby increasing the chances for pollen capture. In aquarium studies, however, pollen of *R. megacarpa* remained mostly near the stigmas of the flower from which it was

released (Van Vierssen et al., 1982), an indication that selfing may occur frequently in the species. In any case, sexual reproduction in *Ruppia* plays a large role in its propagation (Edwards, 1976).

Information on the reproductive biology of the marine *Posidonia* is too general to infer much of its breeding system. The flowers consist of three or four sessile anthers surrounding the simple carpel, which terminates in a feathery, lacerate stigma (Sculthorpe, 1967). When the anthers dehisce, clouds of filamentous pollen are released into the water (Pettitt et al., 1981). Because the carpel is surrounded by anthers, the potential for autogamy might be quite high. Without direct observations and knowledge of possible dichogamy, however, this conclusion can only be implied.

In monoecious plants, autogamy is prevented but geitonogamy is not. Hartog (1970), however, attributed cross-fertilization to all monoecious seagrasses (*Zostera, Heterozostera, Halophila decipiens*) due to their protogyny.

In the monoecious *Zostera marina*, the potential for xenogamy is indeed increased by protogyny (Arber, 1920). Pollen is shed in cloudy masses, with pollen tubes already beginning to protrude (Arber, 1920). Pollen is released either slowly under water or quickly in floating masses, which readily adhere to any object coming in contact with the grains (De Cock, 1980). Despite widespread protogyny in *Zostera* (Sculthorpe, 1967), the proximity of male and female flowers within a spathe (Aston, 1973) suggests the possibility of occasional geitonogamy among flowers within the spathe. In fact, De Cock (1980) observed that self-pollination in *Zostera* will occur in the absence of cross-pollination and takes place widely in plants cultured in aquariums. The extent of geitonogamy in natural populations of *Zostera* is not clearly known.

A similar situation exists in the related genus *Heterozostera*. Although male and female flowers occur within the same spathe, the flowers are protogynous, with the stigmas falling off prior to the opening of anthers within the same spadix (Aston, 1973; Hartog, 1970). *Halophila decipiens*, the sole monoecious species of the principally dioecious genus, is likewise protogynous, with male and female flowers occurring within the same spathe (Hartog, 1970).

In the monoecious *Ceratophyllum demersum*, xenogamy is probably infrequent. The rarity of flowering and the aggressive vegetative growth of this species greatly limit sexual reproduction (Les, 1985). When sexual reproduction occurs, the self-compatibility of the species allows for geitonogamy (Les, 1980, 1985). Although xenogamy is possible,

transport of pollen tends to be within large clones (Les, 1986b).

Flowers of the monoecious *Lepilaena australis* are similar to those of *Zannichellia* but appear to be more conducive to xenogamy. Female flowers are borne on the upper parts of the plant and occur at the ends of peduncles up to 14 cm long. The male flowers are very short-stalked and occur on the lower portions of the plant (Aston, 1973). In this arrangement, geitonogamy would require the upward transport of pollen, and lateral movement of pollen would be more likely to facilitate xenogamy. In monoecious *Lepilaena preissii*, however, the male and female flowers are clustered together (Aston, 1973) and here geitonogamy is more likely. Pollination in the monoecious *Lepilaena cylindrocarpa* is ephydrophilous. Stigmas create depressions in the water surface into which floating pollen grains released from submersed anthers were drawn (Van Vierssen et al., 1982). This arrangement would facilitate geitonogamy (as pollen from the same plant would be in closest proximity to the stigmas), although xenogamy may occur as well.

The freshwater genus *Najas* is predominantly monoecious (with only one dioecious species) and annual (Haynes, 1977). The pollination biology of *Najas* is not known in detail, but several aspects have been described. Sculthorpe (1967) observed that pollen tube germination is precocious, often as the microspores are released from the floral envelope. He reasoned that the dense growth of plants would place male and female flowers together, and that liberated pollen would be (p. 301) ". . . caught haphazardly on the elongated stigmas." Aston (1973) observed that pollen is discharged apically through an opening in the floral envelope and is transported through the water to the stigmas. There is no indication that *Najas* possesses any mechanisms to prevent geitonogamy.

Dioecy is the only sexual condition that ensures xenogamy, and all dioecious hydrophiles must be regarded as possessing a high outcrossing potential. Dioecy does not guarantee outcrossing, however, which will occur only when all conditions have been satisfied.

THE EXTENT OF GENETIC VARIATION IN HYDROPHILE POPULATIONS

The genetic structure of a hydrophile population ultimately determines the extent of outcrossing. In sexual and xenogamous populations, outcrossing will occur whenever sexual reproduction is consummated between genetically different individuals. How different genetically are individuals in

TABLE 2. *Flowering frequency and short-shoot sex ratios for five populations of* Thalassia testudinum. *M = male; F = female (adapted from Durako & Moffler, 1985a).*

Sites	Flowering Frequency	Sex Ratio M : F
Cockroach Bay	38%	1 : 1
Egmont Key	26%	1.7 : 1
Big Coppit Key	25%	1 : 3.1
Lassing Park	22%	1.4 : 1
No Name Key	3%	2.0 : 1

TABLE 3. *Flowering frequency and plant sex ratios in three species of dioecious* Elodea; *F = female, M = male (computed from data in Catling & Wojtas, 1986 (A); Cook & Urmi-König, 1985 (B)).*

Species	% in Flower	Sex Ratio F : M
A. *Elodia bifoliata*	53	1.8 : 1
B. *E. bifoliata*	87	1.2 : 1
A. *Elodea nutallii*	32	1.6 : 1
B. *E. nutallii*	—	1.2 : 1
A. *Elodea canadensis*	32	1.4 : 1
B. *E. canadensis*	—	1.2 : 1

hydrophile populations? Few published studies have addressed this essential question.

Dioecy and outcrossing are typically equated despite unresolved questions whether heterosexual individuals of dioecious plant species always differ genetically. Although sex in the majority of dimorphic plant species is believed to be determined solely by genetic factors (Lloyd & Bawa, 1984), diphasic responses have been reported in many dioecious plants, including species reported to have sex chromosomes (Freeman et al., 1980). If diphasic sex changes can occur in hydrophiles that reproduce extensively by vegetative growth, then there would be at least the potential for clonally derived individuals to express different sexes. Unfortunately, there is little available evidence to provide a satisfactory resolution to the question of whether such changes occur in dioecious hydrophiles or not.

In addition to direct observation of population sexuality over several seasons, Lloyd & Bawa (1984) regarded consistent sex production in widely spaced ramets or branches and sex ratios of unity as circumstantial evidence of phase stability in dioecious plants. Few studies of dioecious hydrophiles have provided direct observation of phase stability or change. Grey & Moffler (1978) reported an overall female-biased sex ratio of 3 : 1 for *Thalassia testudinum*, although they observed a range of ratios from 21 : 0 to 1 : 1. Durako & Moffler (1985a) determined that three out of four populations of *T. testudinum* were characterized by male-biased ratios (Table 2). Further studies of *Thalassia* demonstrated that male-biased ratios occurred only in one of three years of observation and correlated with highest seed output (Durako & Moffler, 1985b). There was no apparent relation of the ratios to flowering frequency; male bias occurred at flowering frequencies from 3–38% (Table 2). In *Thalassia testudinum*, the variation of sex ratios among populations and their deviation from unity

may indicate that sex expression in this species is affected by environmental conditions. Durako & Moffler (1985a, b), however, attributed the yearly differences in the sex ratios not to sex change but to annual variation in the density of males; the female densities remained fairly constant temporally.

Biased sex ratios also occur in other dioecious hydrophile species. Estimates of flowering frequency and sex ratios computed from data published by Cook & Urmi-König (1985) and Catling & Wojtas (1986) for three dioecious and ephydrophilous species of *Elodea* indicate female-biased ratios of 1.2–1.8 : 1 (Table 3). In *Elodea*, the highest female bias was found in *E. bifoliata*, the most frequently flowering species (Table 3). Greater female-biased sex ratios occur in dioecious marine hydrophiles, epitomized by *Phyllospadix*, with an approximately 12 : 1 ratio of female to male plants (Dawson, 1966). Using specimen lists cited in Hartog (1970), flowering frequencies and sex ratios were computed for seven dioecious species of hydrophilous seagrasses (Table 4). There are some indications of stability in the sex ratios of these species. The 11 : 1 ratio calculated for *Phyllospadix* approximates the 12 : 1 ratio reported independently by Dawson (1966); both the frequency of flowering and the sex ratio are nearly equal for two species of *Syringodium*. Although sex ratios of three species of *Elodea* are similar, Cook & Urmi-König (1985) have discussed various aspects of sex instability in the genus. Sex ratios of unity were noted only in *Thalassia testudinum*, *Halodule uninervis*, and *Thalassodendron ciliatum*; most other ratios were female-biased (Tables 3, 4). An interesting trend in the data is a possible relationship between sex ratios and flowering frequency. A decrease in the frequency of flowering is associated with a lower female bias in the sex ratios; lower floral frequencies appear to be asso-

TABLE 4. *Flowering frequency and plant sex ratios in seven dioecious marine hydrophiles; F = female, M = male (computed from data in Hartog, 1970).*

Species	% in Flower (seasonal)	Sex Ratio F:M
Phyllospadix scouleri	83	11:1
Phyllospadix torreyi	78	4.2:1
Syringodium filiforme	39	1.4:1
Syringodium isoetifolium	38	1.3:1
Halodule uninervis	18	1:1
Thalassodendron ciliatum	12	1:1
Cymodocea nodosa	12	1:3

TABLE 5. *Sex ratios in a population of* Ceratophyllum demersum *from Okauchee Lake, Wisconsin, U.S.A. M = male; F = female; * = female-biased.*

Plant Number	Number of Male Flowers	Number of Female Flowers ( ) = fruits	Plant Sex Ratio (M:F)	Average Combined Sex Ratio (M:F)
1	22	3	7.3:1	7.3:1
2	0	2	0:2*	4.4:1
3	0	1 (1)	0:2*	3.1:1
4	15	2	7.5:1	4.1:1
5	12	5	2.4:1	3.5:1
6	51	6	8.5:1	5.0:1
7	7	9	0.8:1*	3.7:1
8	39	10	3.9:1	3.7:1
9	14	9	1.6:1	3.3:1
10	1	2	0.5:1*	3.2:1
11	1	0	1:0	3.2:1
12	15	7	2.1:1	3.1:1
13	7	3	2.3:1	3.1:1
14	3	8 (3)	0.3:1*	2.6:1
15	1	0	1:0	2.7:1
16	1	2	0.5:1*	2.6:1
17	10	1	10.0:1	2.7:1
18	5	3	1.7:1	2.7:1
19	0	6	0:6*	2.5:1
20	0	5	0:5*	2.3:1
21	2	5	0.4:1*	2.2:1
22	6	1	6.0:1	2.3:1
23	10	5	2.0:1	2.2:1
24	28	38	0.7:1*	1.8:1
25	24	12	2.0:1	1.8:1
26	6	7	0.9:1*	1.8:1
27	16	5	3.2:1	1.8:1
28	26	10	2.6:1	1.9:1
29	12	6	2.0:1	1.9:1
30	12	7	1.7:1	1.9:1
Total	346	184	—	1.9:1

ciated with equal or male-biased ratios (Table 4). This trend is also apparent in *Elodea*, but to a much lesser degree (Table 3). Sex ratios of the dioecious *Thalassodendron ciliatum* indicate a male bias at low flowering frequencies of 15–19% (Kay, 1971). *Syringodium* flowers commonly, and produces mostly female flowers (Kay, 1971). An exception to this trend occurs in the infrequently flowering genus *Cymodocea*, which has been observed to produce only female flowers (Kay, 1971). The absence of male flowers, however, was possibly due to the sampling of a single clonal population (Kay, 1971). In other populations of *Cymodocea*, however, sexes co-occur but are partitioned spatially into unisexual zones (Caye & Meinesz, 1985). It is possible that the relationship of reduced female-bias and low flowering frequency may result in part from sampling error.

Sex-ratio data must be interpreted cautiously. The different methods of assessment (e.g., plant ratios, short-shoot ratios, single population ratios, multiple population ratios) and wide variability in sample size are only two shortcomings. Furthermore, there have been no real efforts to distinguish between genets and ramets in populations where studies have been conducted. Because of the effective system of vegetative reproduction in hydrophiles, sex-ratio data may be misleading. Apomixis and other factors that can influence sex ratios have been summarized elsewhere (e.g., Opler & Bawa, 1978). Ultimately, a precise knowledge of sex expression in dioecious hydrophiles will be required before any reliable conclusions can be drawn from sex-ratio data.

Less is understood of sex ratios in monoecious hydrophiles. In *Ceratophyllum demersum*, plants fruit rarely, but fertile specimens are typically male-biased (Les, unpubl.). Sex ratios computed for a fertile population of *C. demersum* from Wisconsin, U.S.A., provide some insights into its sex ratios. The ratios were calculated by counting all flowers on 30 dissected plants. The range of sex-ratio variation in this population is extensive. Most plants are male-biased (up to 10:1); however, nearly a third of the sample is female-biased (Table 5). Although the species is monoecious, unisexual plants were observed for both sexes. The overall sex ratio was male-biased at 1.9:1. Fruiting was low (2%), and fruits were found only on plants with female-biased ratios. In species of *Ceratophyllum* where fruiting is more common, sex ratios appear to be less male-biased (Les, unpubl.). Reasons for the

wide amplitude of sex distribution on *Ceratophyllum* plants are not clear, but it is apparent that sex ratios are not rigidly fixed genetically. The potential for environmental influence of sex expression in *Ceratophyllum* was demonstrated by the sole production of male flowers on plants of *C. demersum* and *C. echinatum* grown under continuous illumination (Les, 1980). Plants from the same population observed in the field were normally monoecious.

In *Zostera marina*, a consistent sex ratio of one pistil to one anther was observed from a population in France, despite wide variability in the flower number per spathe (Jacobs & Pierson, 1981). In monoecious *Zannichellia*, the close association of male and female flowers indicates a probable 1 : 1 ratio. Such consistent values may indicate tighter genetic regulation of sex expression in these species. Sex ratios of other monoecious hydrophiles have not been studied in any detail.

Although hydrophily is often equated with outcrossing, a critical point is made by Faegri & van der Pijl (1979: 41): ". . . hydrophilous mechanisms give no guarantee against autogamy. However, the gregarious habit of the plants in question will generally cause allogamy and counteract auto- and geitonogamy unless *the whole meadow represents a single clone*" [emphasis mine]. The extent of clonal growth in hydrophiles is surely an important determinant of their population structure.

Hutchinson (1975) recognized that most reproduction in hydrophytes is asexual, and as a result (p. 238) ". . . large clonal populations are likely to be very common in lacustrine angiosperms." He also observed that for perennial species (p. 233), ". . . any given specimen is much more likely to have arisen by asexual than by sexual processes."

An important difference between asexual reproduction in terrestrial plants and aquatic plants is that vegetative buds, fragments, turions, etc. in the latter can facilitate dispersal without the planting requirement necessary for fragments of most terrestrial species (Hutchinson, 1975). This feature is evidently one of the most important mechanisms for dispersal in aquatic plants, particularly those that produce small quantities of seeds. The efficiency of vegetative dispersal in hydrophilous species is exemplified by the spread of *Elodea canadensis* across Europe, which occurred within 50 years following its introduction from an uncertain North American source. This feat was accomplished entirely by vegetative means, as the species is dioecious and (with one exception) only female plants were introduced (Cook & Urmi-König, 1985; Grant,

1981). Cook & Urmi-König (1985: 118) remarked that the ". . . aggressive vegetative growth [of *Elodea*] leads to . . . the consequence that one habitat becomes fully occupied by one genotype."

Similar conclusions have been drawn for other hydrophiles. In *Cymodocea serrulata*, the "only" means of medium- or long-range dispersal is by vegetative fragments, and clonal growth patterns are typical due to the vigorous vegetative growth (Kay, 1971). In *C. nodosa*, seeds are not disseminated, and sexually derived offspring must be spatially and temporally restricted (Caye & Meinesz, 1985). Likewise, vegetative reproduction is more prevalent than sexual reproduction in the maintenance and spread of *Thalassia* (Kay, 1971; Grey & Moffler, 1978). In *Halophila*, *Thalassia*, *Cymdocea*, and *Halodule*, sexual reproduction is rare and reproduction is mostly vegetative (Edwards, 1976; Jacobs & Dicks, 1985). For *Halophila hawaiiana*, Herbert (1986: 101) stated that ". . . it is possible that the monospecific meadows of *Halophila* in the Hawaiian islands are made up of plant material from a single genetic individual." According to Obermeyer (1966), propagation of *Halophila ovalis* is mainly vegetative and may lead to the formation of "homogeneous colonies." *Zostera marina* reportedly flowers infrequently and maintains stable population sizes by vegetative reproduction (Harrison, 1979). Both *Zostera* and *Heterozostera* reproduce asexually by unusual vegetative propagules (Cambridge et al., 1983). *Ceratophyllum demersum* is rare-flowering and highly clonal (Les, 1986b). Haynes & Holm-Nielsen (1987) stated that all Zannichelliaceae "grow clonally."

Widespread vegetative growth in hydrophilous species may lead to the formation of large, genetically uniform populations in which outcrossing would be thwarted even among sexual, xenogamous individuals. Hutchinson (1975), however, noted that the few aquatic annuals such as *Najas* are exceptions to the generalization that most hydrophytes reproduce asexually. Development of an annual habit in hydrophiles, where consistently high levels of seed production are essential for survival, may have provided an escape from constraints on outcrossing imposed by dense clonal growth. Although vicinism may also occur in diclinous annuals such as *Najas*, there is at least a much higher potential for dispersal of sexually derived propagules to other sites, which betters the chances that future generations will breed with genetically different individuals.

The foregoing discussion cites only circumstan-

TABLE 6. *Summary of enzyme variability in species from 13 hydrophilous angiosperm genera.* * = *enzyme displaying intra- or interpopulational variability at one or more loci; all other enzymes monomorphic (compiled from A: McMillan et al., 1981; B: Les, 1986b; C: McMillan, 1981; D: McMillan & Williams, 1980; E: McMillan, 1982; F: Triest et al., 1986; G: McMillan & Phillips, 1981; H: Gagnon et al., 1980).*

Species	Enzymes Surveyed
Amphibolis antarctica (A)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
A. griffithii (A)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
Ceratophyllum demersum (B)	APH, ADH, EST, GOT, G-6-PGD, GDH, IDH, LAP, MDH, PGI, PGM
Cymodocea rotundata (C)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
C. serrulata (C)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
Halodule pinifolia (A)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
H. uninervis (A)	APH, EST*, G-6-PD, GOT, MDH, PER, PGI, PGM
H. wrightii (A)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
Halophila decipiens (D)	APH, GDH, GOT, MDH, PER, PGI, PGM
H. hawaiiana (D)	APH, GDH, GOT, MDH, PER, PGI, PGM
H. johnsonii (D)	APH, GDH, GOT, MDH, PER, PGI, PGM
H. minor (D)	APH, GDH, GOT, MDH, PER, PGI, PGM
H. ovalis (A)	APH, EST, GDH, GOT, MDH, PER, PGI, PGM
H. stipulacea (D)	APH, GDH, GOT, MDH, PER, PGI, PGM
H. sp. (A)	APH, EST, GDH, GOT, MDH, PER, PGI, PGM
Heterozostera tasmanica (E)	APH, G-6-PD, GOT, MDH, PER, PGI, PGM
Najas marina (F)	ADH*, ME*, SkDH, XDH
Posidonia australis (A)	APH, EST, GDH, GOT, MDH, PER, PGI, PGM
P. sinuosa (A)	APH, EST, GDH, GOT, MDH, PER, PGI, PGM
Phyllospadix scouleri (G)	ADH*, APH, EST, G-6-PD, GDH, GOT, MDH, PER, PGI, PGM
P. serrulatus (G)	ADH, APH, EST, G-6-PD, GDH, GOT, MDH, PER, PGI, PGM
P. torreyi (G)	ADH, APH, EST, G-6-PD, GDH, GOT, MDH, PER, PGI, PGM
Syringodium filiforme (E)	APH, G-6-PD, GOT, MDH, PER, PGI, PGM
S. isoetifolium (E)	APH, G-6-PD, GOT, MDH, PER, PGI, PGM
Thalassia hemprichii (E)	APH, G-6-PD, GOT, MDH, PER, PGI, PGM
T. testudinum (E)	APH, G-6-PD, GOT, MDH, PER, PGI, PGM
Thalassodendron ciliatum (E)	APH, EST, G-6-PD, GOT, MDH, PER, PGI, PGM
Zostera capensis (E)	ADH, APH, GOT, MDH, PER, PGI, PGM
Z. capricorni (E)	APH, ADH, GOT, MDH, PER, PGI, PGM
Z. marina (H)	ADH, CAT, DIA, G-6-PD, G-6-PGD, GDH, GTR, GOT*, HK, IDH, MDH, PGI*, PGM, PMI, SDH, SOD
Z. marina (E)	APH, GOT, MDH*, PER, PGI, PGM*
Z. muelleri (E)	APH, ADH, GOT, MDH, PER, PGI, PGM
Z. novazelandica (E)	ADH, APH, GOT, MDH, PER, PGI, PGM

tial evidence for assessing the degree of genetic variation in hydrophile populations; however, there is some empirical evidence that can be brought to bear on this issue. The use of enzyme gel electrophoresis has allowed for the direct estimation of genetic variation in plant populations (Gottlieb, 1981; Brown, 1979; Hamrick et al., 1979). Fortuitously, most electrophoretic studies on aquatic plants have been carried out with seagrasses (Wain et al., 1985), all of which are hydrophilous.

Approximately 30 species representing 12 genera of seagrasses have been studied electrophoretically (Wain et al., 1985). These analyses consistently report genetic uniformity and provide little evidence of electrophoretically detectable genetic variation in hydrophile populations (Table 6). According to Wain et al. (1985: 43), "In most species [of seagrasses] there exists no intraspecific variation in banding patterns, even across large geographic distances." Furthermore, the level of heterozygosity in seagrasses is apparently extremely low (Wain et al., 1985; McMillan, 1982). Of the 32 hydrophile species studied, enzyme variability has been reported only in *Zostera marina*, *Halodule uninervis*, *Phyllospadix scouleri*, and *Najas marina* (Table 6). As Crawford (1983) discussed, enzyme uniformity of this sort is more characteristic of self-pollinating plants than of outcrossers.

Preliminary studies of *Ceratophyllum* have giv-

en similar results. Populations of the rare-flowering *C. demersum* are relatively uniform both morphologically and genetically, and are probably clonal (Les, 1988b). Populations of the more sexual species *C. echinatum* are more variable morphologically and display patterns of intrapopulational variability that are quite representative of the species as a whole (Les, 1988a). An electrophoretic study of populations of both species is under way in hopes of providing further insight into the genetic structure of populations of the predominantly sexual vs. asexual species of *Ceratophyllum*.

Enzyme polymorphisms have been reported in electrophoretic studies of the annual species *Najas marina* (Triest et al., 1986), a possible indication of greater genetic diversity in this genus. Further electrophoretic surveys are necessary to determine the extent of intrapopulational genetic variation in other *Najas* species and annual hydrophiles in general. An informative study would be to compare populations of *Najas* and *Zannichellia* electrophoretically. Although both genera are annual, *Zannichellia* has few species compared with *Najas*, a possible outcome of the predominantly geitonogamous/autogamous breeding system of *Zannichellia*.

Despite the large number of electrophoretic studies carried out on hydrophiles, an adequate understanding of population structure in this group is far from being reached. A major difficulty has been that most studies have reported genetic data qualitatively rather than quantitatively (Table 6). Because data have not been presented in allelic form, it is not possible to compute appropriate quantitative measurements of population structure such as fixation indices, average heterozygosity, proportion of loci polymorphic, number of alleles/locus, and gene diversities (Nei, 1987). Furthermore, no study has measured outcrossing rates quantitatively in any hydrophilous species. Because models for estimating outcrossing rates should be evaluated in terms of the pollination system of a species (Shea, 1987), it may first be necessary to develop a specific model for hydrophilous plants. Electrophoretic approaches provide a powerful means of estimating population structure and outcrossing rates. The scarce amount of genetic variation detected in hydrophiles thus far is an impetus for continuing investigations in this area.

A satisfactory resolution to the question of population structure in hydrophiles will not come until rigorous genetic analyses have been carried out. On the other hand, it is important to emphasize that the widely held assumption of extensive genetic variability in hydrophile populations is not sup-

ported by available data. The high degree of clonal growth attributed to hydrophiles may result in genetically uniform populations. Even in instances where sex expression of dioecious hydrophiles may be under strict genetic control, clonal growth allows for the possibility of the establishment of extensive unisexual populations. Because of these factors, clonal growth may thwart outcrossing in many hydrophiles. In support of this conclusion are preliminary electrophoretic data which indicate little detectable genetic variation in hydrophile populations.

Assumptions that hydrophiles are outcrossing and produce genetically variable offspring should not be taken for granted. The inefficiency of hydrophily, reduced sexuality, of autogamy and geitonogamy, and widespread clonal growth have the potential to restrict outcrossing greatly in this group. Because no studies have characterized genetically the actual level of inbreeding vs. outcrossing in any particular hydrophyte species, however, it is premature to argue too strongly either for or against the first component of the outcrossing hypothesis.

COSTS VS. BENEFITS OF DICLINY IN HYDROPHILES

The second component of the outcrossing hypothesis, that outcrossing in hydrophiles offsets evolutionary costs associated with transitions to dicliny, is even more difficult to assess. One difficulty with pursuing this possibility is that it remains to be proven conclusively that hydrophiles are highly outcrossing.

Plant breeding systems involve three general mechanisms: inbreeding, outcrossing, and apomixis (Briggs & Walters, 1984). Historically, outcrossing has been associated with enhanced genetic variability and heterozygosity, whereas inbreeding and obligate apomixis are related to low genetic variability (Faegri & van der Pijl, 1979; Briggs & Walters, 1984). With adapted gene complexes preserved by well-developed asexual reproduction systems, it is reasonable to assume that it would be advantageous for sexual reproduction in hydrophiles to provide a means of outcrossing that could respond facultatively to changing environmental conditions. Because dicliny may promote or enforce xenogamy, the prevalence of the unisexual condition in hydrophiles has been linked to outcrossing and widespread genetic variability. Presumably, a rich gene pool would facilitate adaptation and response to environmental changes. Therefore, enhanced genetic variation is interpreted as a major possible benefit of dicliny.

Grant (1975) pointed out the liability of ineffi-

cient sexual reproduction in diclinous plants, a cost related to reduced seed output in populations. If sex ratios are equal, half of the flowers would not bear seeds in either monoecious or dioecious species. This outcome could have serious consequences in hydrophiles in which seed output is suppressed by other factors such as infrequent sexual reproduction and extensive vegetative growth. Female-biased or equal sex ratios, however, may maximize seed production in dioecious species (Grant, 1975; Opler & Bawa, 1978). As discussed above, female-biased sex ratios have been noted in *Elodea*, *Phyllospadix*, *Syringodium*, and *Thalassia*, and ratios of unity have been found in *Halodule* and *Thalassodendron*. It is important to emphasize that female-biased sex ratios do not always alleviate problems of low seed production. A surplus of females in a population (in favor of reduced males) may also result in low seed output because of inadequate pollination (Grant, 1975). This limitation may have been responsible for a 28% loss in seed set observed in a population of the monoecious *Zostera marina* (Churchill & Riner, 1978). A compromise between seed output and adequate pollination may be reflected in male-biased sex ratios reported in *Ceratophyllum*, *Cymodocea*, and *Thalassia*.

Low seed output in hydrophiles may be specially implicated in their dispersal mode. Dispersal has been described as the physical basis of gene flow and in most plants occurs via transport of pollen or fruits (Grant, 1981). In aquatic plants, dispersal of vegetative propagules is also of great importance. In hydrophiles, reduced seed output may result in a greater dependence on the transport of pollen and vegetative propagules as avenues of dispersal and gene flow. This relationship may be quite significant for freshwater hydrophiles where pollen gene flow is restricted to the single body of water in which the population occurs. In such instances, interpopulational gene flow may be entirely by transport of vegetative propagules. In contrast to perennial species, seed output of diclinous annual hydrophiles is extremely high, reaching levels of 100 seeds/m² in *Lepilaena* (Vollebergh & Congdon, 1986). Evidently, adaptation to the annual habit has overcome seed limit costs of dicliny. In some diclinous annuals, such as monoecious *Zannichellia*, however, seed set is ensured by geitonogamy (Haynes & Holm-Nielsen, 1987), thereby negating any possible advantages of outcrossing associated with monoecy.

These circumstantial data provide no satisfying resolution to the question of whether or not the costs of dicliny are outweighed by its selective advantages. Better insight into this matter may be gained by studying the relative fitness of closely related species in genera such as *Zannichellia* that possess monoecious, dioecious, and hermaphroditic sexual conditions.

EXPLANATIONS FOR THE EVOLUTION OF DICLINY IN HYDROPHILES

The third component of the outcrossing hypothesis is that dicliny evolved in hydrophiles principally as a mechanism of promoting outcrossing. This element of the hypothesis can be challenged outright because dicliny does not always guarantee outcrossing. Lewis (1979: 4) emphasized that ". . . separation of sexes . . . offers no protection against sib-mating, because pollen from a male plant is equally effective on a female whether it be a sib or non-sib," and that ". . . separation of sexes has a limited value as an outbreeding device in static plants." It is also important to realize that inbreeding is not restricted to hermaphroditic species but can also result in diclinous species as a result of geitonogamy, and possibly even from xenogamy due to vicinism (Grant, 1981). Furthermore, anomalies such as geitonogamy in monoecious *Zannichellia* make it difficult to accept outcrossing as the compelling force in the evolution of dicliny.

Some consideration has been given to other factors influencing sexual conditions in plants such as relative resource allocation to maternal and paternal function, and constraints imposed by the dynamics of the pollination system (Bawa & Beach, 1981). Unfortunately, these discussions have not considered water-pollinated plants.

If outcrossing has been important in the evolution of hydrophiles, then why are so many species characterized by infrequent sexual reproduction? On the other hand, if outcrossing has not been evolutionarily important in this group, then why has dicliny been conserved? This dilemma may be resolved by taking into account the early evolution of hydrophilous plants. Kimura & Ohta (1971) observed that the extent of genetic variability derived from sexual recombination could be attained by mutation alone given sufficient time, and they emphasized that gene recombinations are broken up by sexual recombination as quickly as they are made. They believed that the greatest advantage of sexual reproduction is to enhance the rate of evolution.

Although the process of adaptation accompanying the transition to hydrophily may have occurred slowly by mutation alone, the combination of sexual recombination and an outcrossing breed-

ing system would have facilitated the process by generating pools of genetic variability more rapidly. In this way, the ancestors of modern hydrophiles may have benefited greatly from sexuality and the outcrossing potential conferred by dicliny, especially if their progenitors lacked other mechanisms to prevent autogamy. In species where hydrophily was derived mainly from lines of self-compatible anemophilous plants, dicliny may have preceded hydrophily.

Once species had become well adapted to hydrophily and a submersed existence, greater fitness may have resulted from maintaining genetically uniform rather than highly variable offspring. Because aquatic environments are relatively constant, vegetative growth would maintain adaptive gene complexes and prevent their breakup by sexual reproduction. The phasing out of sexual reproduction may have been accompanied by greater resource allocation to vegetative growth. A shift to asexual reproduction would likely result in slow evolutionary rates and low species diversity in the group, precisely the pattern that we see in the fossil record of several modern species. By this scenario, dicliny in extant hydrophiles is essentially a relictual condition of uncertain consequence to their present reproductive biology. There are additional appealing aspects of this interpretation. Because dispersal in rare-flowering perennial hydrophiles is likely to be predominantly by vegetative propagules, many populations would be expected to be clonal and genetically uniform. The available data show that these patterns commonly occur in water-pollinated plants. The maintenance of sexuality in extant annual hydrophiles may account for their higher species diversity, the result of accelerated evolutionary rates associated with sexual recombination and outcrossing.

SUMMARY

Because of the prevalence of dicliny in hydrophiles, it is widely believed that water pollination in aquatic plants is linked to an outcrossing breeding system. Actually, there is little evidence that three necessary conditions for outcrossing (sexuality, xenogamy, genetically variable populations) are met by many extant species. Interrelationships of inefficient pollen transfer, reduced sexuality, widespread clonal growth, and diminished seed production in hydrophiles may reflect a major adaptive shift towards asexuality as a means of preserving adaptive gene complexes in stable aquatic environments. This hypothesis explains the apparent slow evolutionary rates and low species diversity

of some hydrophilous species noted in the fossil record, as well as the greater species diversity observed in sexual, annual, potentially outcrossing species. If this interpretation is correct, then dicliny persists in modern perennial hydrophiles as a relictual condition associated with their early evolution.

Although the course of evolution in water-pollinated plants does not appear to differ fundamentally from that of other plants, the complex interactions of hydrophily and other aspects of their reproductive biology are likely to have profoundly influenced the patterns of their present diversity. As we improve our methods of evaluating the breeding systems and population structure of hydrophilous species, so will our understanding of evolution in this unusual group of plants be expanded.

LITERATURE CITED

ACKERMAN, J. D. 1983. Current flow around *Zostera marina* plants and flowers: implications for submarine pollination. Biol. Bull. Mar. Biol. Lab. Woods Hole 165: 504.

———. 1986. Mechanistic implications for pollination in the marine angiosperm *Zostera marina*. Aquatic Bot. 24: 343–353.

AGAMI, M., S. BEER & Y. WAISEL. 1986. The morphology and physiology of turions in *Najas marina* L. in Israel. Aquatic Bot. 26: 371–376.

ARBER, A. 1920. Water Plants. A Study of Aquatic Angiosperms. Reprint, J. Cramer, Lehre.

ASTON, H. I. 1973. Aquatic Plants of Australia. Melbourne Univ. Press.

BAWA, K. S. 1980. Evolution of dioecy in flowering plants. Ann. Rev. Ecol. Syst. 11: 15–39.

——— & J. H. BEACH. 1981. Evolution of sexual systems in flowering plants. Ann. Missouri Bot. Gard. 68: 254–274.

BRIGGS, D. & S. M. WALTERS. 1984. Plant Variation and Evolution, 2nd edition. Cambridge Univ. Press, New York.

BROCK, M. A. 1982. Biology of the salinity tolerant genus *Ruppia* L. in saline lakes in south Australia. II. Population ecology and reproductive biology. Aquatic Bot. 13: 249–268.

BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. Theor. Populat. Biol. 15: 1–42.

CAMBRIDGE, M. L., S. A. CARSTAIRS & J. KUO. 1983. An unusual method of vegetative propagation in Australian Zosteraceae. Aquatic Bot. 15: 201–203.

CATLING, P. M. & W. WOJTAS. 1986. The waterweeds (*Elodea* and *Egeria*, Hydrocharitaceae) in Canada. Canad. J. Bot. 64: 1525–1541.

CAYE, G. & A. MEINESZ. 1985. Observations on the vegetative development, flowering and seeding of *Cymodocea nodosa* (Ucria) Ascherson on the Mediterranean coasts of France. Aquatic Bot. 22: 277–289.

——— & ———. 1986. Experimental study of seed germination in the seagrass *Cymodocea nodosa*. Aquatic Bot. 26: 79–87.

CHURCHILL, A. C. & M. I. RINER. 1978. Anthesis and

seed production in *Zostera marina* L. from Great South Bay, New York, U.S.A. Aquatic Bot. 4: 83–93.

COOK, C. D. K. 1982. Pollination mechanisms in the Hydrocharitaceae. Pp. 1–15 *in* J. J. Symoens, S. S. Hooper & P. Compère (editors), Studies on Aquatic Vascular Plants. Roy. Bot. Soc. Belgium, Brussels.

——— & K. URMI-KÖNIG. 1985. A revision of the genus *Elodea* (Hydrocharitaceae). Aquatic Bot. 21: 111–156.

———, B. J. GUT, E. M. RIX, J. SCHNELLER & M. SEITZ. 1974. Water Plants of the World. W. Junk, The Hague.

COX, P. A. 1983. Search theory, random motion, and the convergent evolution of pollen and spore morphology in aquatic plants. Amer. Naturalist 121: 9–31.

CRAWFORD, D. J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. Pp. 257–287 *in* S. D. Tanksley & T. J. Orton (editors), Isozymes in Plant Genetics and Breeding, Part A. Elsevier Science Publishers, Amsterdam.

DAWSON, E. Y. 1966. Marine Botany. An Introduction. Holt, Rinehart & Winston, New York.

DE COCK, A. W. A. M. 1980. Flowering, pollination and fruiting in *Zostera marina* L. Aquatic Bot. 9: 201–220.

DURAKO, M. J. & M. D. MOFFLER. 1985a. Observations on the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae) II. Leaf width as a secondary sex character. Aquatic Bot. 21: 265–275.

——— & ———. 1985b. Observations on the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae) III. Spatial and temporal variations in reproductive patterns within a seagrass bed. Aquatic Bot. 22: 265–276.

——— & ———. 1987. Factors affecting the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae). Aquatic Bot. 27: 79–95.

EDWARDS, P. 1976. Illustrated Guide to the Seaweeds and the Seagrasses in the Vicinity of Port Aransas, Texas. Univ. Texas Press, Austin, Texas.

FAEGRI, K. & L. VAN DER PIJL. 1979. The Principles of Pollination Ecology, 3rd edition. Pergamon Press, New York.

FREEMAN, D. C., K. T. HARPER & E. L. CHARNOV. 1980. Sex change in plants: old and new observations and new hypotheses. Oecologia 47: 222–232.

GAGNON, P. S., R. L. VADAS, D. B. BURDICK & B. MAY. 1980. Genetic identity of annual and perennial forms of *Zostera marina* L. Aquatic Bot. 8: 157–162.

GAMERRO, J. C. 1968. Observaciones sobre la biología floral y morfología de la Potamogetonácea *Ruppia cirrhosa* (Petag.) Grande. Darwiniana 14: 575–608.

GOTTLEIB, L. D. 1981. Electrophoretic evidence and plant populations. Progr. Phytochem. 7: 1–45.

GRANT, V. 1975. Genetics of Flowering Plants. Columbia Univ. Press, New York.

———. 1981. Plant Speciation, 2nd edition. Columbia Univ. Press, New York.

GREY, W. F. & M. D. MOFFLER. 1978. Flowering of the seagrass *Thalassia testudinum* (Hydrocharitaceae) in the Tampa Bay, Florida area. Aquatic Bot. 5: 251–259.

HAMRICK, J. L., Y. B. LINHART & J. B. MITTON. 1979. Relationships between life-history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10: 173–200.

HARPER, J. L. 1977. Population Biology of Plants. Academic Press, New York.

HARRISON, P. G. 1979. Reproductive strategies in intertidal populations of two co-occurring seagrasses (*Zostera* spp.). Canad. J. Bot. 57: 2635–2638.

HARTOG, C. DEN. 1970. The Sea-grasses of the World. North-Holland, Amsterdam.

HAYNES, R. R. 1977. The Najadaceae in the Southeastern United States. J. Arnold Arbor. 58: 161–170.

——— & L. B. HOLM-NIELSEN. 1987. The Zannichelliaceae in the Southeastern United States. J. Arnold Arbor. 68: 259–268.

HERBERT, D. A. 1986. Staminate flowers of *Halophila hawaiiana*: description and notes on its flowering ecology. Aquatic Bot. 25: 97–102.

HUTCHINSON, G. E. 1975. A Treatise on Limnology, Volume 3. Limnological Botany. John Wiley & Sons, New York.

JACOBS, R. P. W. M. & B. DICKS. 1985. Seagrasses in the Zeit Bay area and at Ras Gharib (Egyptian Red Sea coast). Aquatic Bot. 23: 137–147.

——— & E. S. PIERSON. 1981. Phenology of reproductive shoots of eelgrass, *Zostera marina* L., at Roscoff (France). Aquatic Bot. 10: 45–60.

KAY, Q. O. 1971. Floral structure in the marine angiosperms *Cymodocea serrulata* and *Thalassodendron ciliatum* (*Cymodocea ciliata*). J. Linn. Soc. Bot. 64: 423–429.

KEDDY, C. J. & D. G. PATRIQUIN. 1978. An annual form of eelgrass in Nova Scotia. Aquatic Bot. 5: 163–170.

KIMURA, M. & T. OHTA. 1971. Theoretical Aspects of Population Genetics. Princeton Univ. Press, New Jersey.

LES, D. H. 1980. Contributions to the Biology and Taxonomy of *Ceratophyllum* in the Eastern United States. M.S. Thesis. Eastern Michigan Univ., Ypsilanti, Michigan.

———. 1985. Ceratophyllaceae. Pp. 97–102 *in* G. Leach & P. Osborne (editors), The Freshwater Plants of Papua New Guinea. Univ. Press, Papua New Guinea.

———. 1986a. Systematics and Evolution of *Ceratophyllum* (Ceratophyllaceae): A Monograph. Ph.D. Thesis. Ohio State Univ., Columbus, Ohio.

———. 1986b. The evolution of achene morphology in *Ceratophyllum* (Ceratophyllaceae), I. Fruit-spine variation and relationships of *C. demersum*, *C. submersum*, and *C. apiculatum*. Syst. Bot. 11: 549–558.

———. 1988a. The evolution of achene morphology in *Ceratophyllum* (Ceratophyllaceae), II. Fruit variation and systematics of the "spiny-margined" group. Syst. Bot. 13: 73–86.

———. 1988b. The origin and affinities of the Ceratophyllaceae. Taxon 37: 326–345.

LEWIS, D. 1979. Sexual Incompatibility in Plants. Edward Arnold Press, London.

LLOYD, D. G. 1980. Benefits and handicaps of sexual reproduction. Evol. Biol. 13: 69–111.

——— & K. S. BAWA. 1984. Modification of the gender of seed plants in varying conditions. Evol. Biol. 17: 255–338.

McATEE, W. L. 1939. Wildfowl Food Plants. Their Value, Propagation, and Management. Collegiate Press, Inc., Ames, Iowa.

McCANN, C. 1945. Notes on the genus *Ruppia*. J. Bombay Nat. Hist. Soc. 45: 396–402.

McMILLAN, C. 1976. Experimental studies on flowering and reproduction in seagrasses. Aquatic Bot. 2: 87–92.

———. 1979. Flowering under controlled conditions by *Cymodocea rotundata* from the Palau Islands, Micronesia. Aquatic Bot. 6: 397–401.

———. 1980. Flowering under controlled conditions by *Cymodocea serrulata*, *Halophila stipulacea*, *Syringodium isoetifolium*, *Zostera capensis* and *Thalassia hemprichii* from Kenya. Aquatic Bot. 8: 323–336.

———. 1981. Morphological variation and isozymes under laboratory conditions in *Cymodocea serrulata*. Aquatic Bot. 10: 365–370.

———. 1982. Isozymes in seagrasses. Aquatic Bot. 14: 231–243.

——— & R. C. PHILLIPS. 1981. Morphological variation and isozymes of North American *Phyllospadix* (Potamogetonaceae). Canad. J. Bot. 59: 1494–1500.

——— & S. C. WILLIAMS. 1980. Systematic implications of isozymes in *Halophila* section *Halophila*. Aquatic Bot. 9: 21–31.

———, ———, L. ESCOBAR & O. ZAPATA. 1981. Isozymes, secondary compounds and experimental cultures of Australian seagrasses in *Halophila*, *Halodule*, *Zostera*, *Amphibolis* and *Posidonia*. Austral. J. Bot. 29: 247–260.

MUHLBERG, H. 1982. The Complete Guide to Water Plants. EP Publishing, Ltd.

NEI, M. 1987. Molecular Evolutionary Genetics. Columbia Univ. Press, New York.

OBERMEYER, A. A. 1966. Hydrocharitaceae. Pp. 101–102 *in* Flora of Southern Africa, Volume I. Dept. of Agricultural Technical Services, Pretoria, South Africa.

OPLER, P. A. & K. S. BAWA. 1978. Sex ratios in tropical forest trees. Evolution 32: 812–821.

PETTITT, J. M. & A. C. JERMY. 1975. Pollen in hydrophilous angiosperms. Micron 5: 377–405.

———, S. DUCKER & B. KNOX. 1981. Submarine pollination. Sci. Amer. 244(3): 135–143.

PHILLIPS, R. C., W. S. GRANT & C. P. McROY. 1983.

Reproductive strategies of eelgrass (*Zostera marina* L.). Aquatic Bot. 16: 1–20.

SALISBURY, E. J. 1942. Reproductive Capacity of Plants. G. Bell & Sons, London.

SCULTHORPE, C. D. 1967. The Biology of Aquatic Vascular Plants. Edward Arnold Publishers, London.

SEHGAL, A. & H. Y. MOHAN RAM. 1970. Comparative developmental morphology of two populations of *Ceratophyllum* L. (Ceratophyllaceae) and their taxonomy. J. Linn. Soc. Bot. 82: 343–356.

SHEA, K. L. 1987. Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. Evolution 41: 124–136.

SILVERTOWN, J. W. 1982. Introduction to Plant Population Ecology. Longman, New York.

TALAVERA, S., P. GARCÍA MURILLO & H. SMIT. 1986. Sobre el genero *Zannichellia* L. (Zannichelliaceae). Lagascalia 14: 241–271.

TIFFNEY, B. H. 1981. Fruits and seeds of the Brandon lignite. VI. Microdiptera (Lythraceae). J. Arnold Arbor. 62: 487–516.

TRIEST, L., J. VAN GEYT & V. RANSON. 1986. Isozyme polymorphism in several populations of *Najas marina* L. Aquatic Bot. 24: 373–384.

UOTILA, P., W. VAN VIERSSEN & R. J. VAN WIJK. 1983. Notes on the morphology and taxonomy of *Zannichellia* in Turkey. Ann. Bot. Fenn. 20: 351–356.

VAN VIERSSEN, W. & R. J. VAN WIJK. 1982. On the identity and autecology of *Zannichellia peltata* Bertol. in Western Europe. Aquatic Bot. 13: 367–383.

———, ——— & J. R. VAN DER ZEE. 1982. On the pollination mechanism of some eurysaline Potamogetonaceae. Aquatic Bot. 14: 339–347.

VOLLEBERGH, P. J. & R. A. CONGDON. 1986. Germination and growth of *Ruppia polycarpa* and *Lepilaena cylindrocarpa* in ephemeral saltmarsh pools, Westernport Bay, Victoria. Aquatic Bot. 26: 165–179.

WAIN, R. P., W. T. HALLER & D. F. MARTIN. 1985. Isozymes in studies of aquatic plants. J. Aquatic Pl. Managem. 23: 42–45.

WHITEHEAD, D. R. 1969. Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution 23: 28–35.

YEO, R. R. 1966. Yields of propagules of certain aquatic plants. I. Weeds 14: 110–113.